

Japanese Encephalitis Virus, Okayama *Culex tritaeniorhynchus* (OCT)-541, Line 35-24

Catalog No. NR-91

(Derived from ATCC® VR-343™)

Product Description: Cell lysate and supernatant from hamster kidney (HaK) cells¹ infected with Japanese encephalitis virus (JEV), OCT-541, line 35-24.²

Lot³: 57774149

Manufacturing Date: 16NOV2007

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HaK Cells ¹	Report results	Cell rounding, sloughing, and piling
Identification by Indirect Fluorescent Antibody Assay ⁴	Fluorescence observed	Fluorescence observed
Sequencing of JEV Specific Region (~ 1030 bp)	Consistent with JEV	Consistent with JEV
Titer by TCID ₅₀ Assay ^{5,6} in HaK Cells ¹	Report results	1.6 X 10 ⁷ TCID ₅₀ /mL
RT-PCR Assay of Extracted RNA Using JEV Specific Primers	~ 1145 bp amplicon	~ 1145 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

¹HaK cells: ATCC® CCL-15™.

²The inoculum for NR-91 was ATCC® VR-343™ (Lot: 215629).

³Grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex® 14-471F), 2 mM L-glutamine (Invitrogen™ 25030), and 1 mM sodium pyruvate (Invitrogen™ 11360) for 7 days at 30°C and 5% CO₂.

⁴Using monoclonal antibody reactive with JEV (Millipore MAB8743).

⁵The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁶13 days at 30°C and 5% CO₂.

⁷Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 30 July 2008

Signature: Signature on file

Title: Technical Manager, BEI Authentication or designee

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